ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/119.117

Volume 119, pp. 117–132 *January–March 2012*

Intraornatosporaceae (*Gigasporales*), a new family with two new genera and two new species

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Abstract — A new family (*Intraornatosporaceae*), two new genera (*Intraornatospora*, *Paradentiscutata*), two new species (*P. bahiana*, *P. maritima*), and a new combination (*I. intraornata*) are presented in the *Gigasporales*. The genera, both with diagnostic introverted ornamentations on the spore wall, are distinguished by spore wall structure and germ shield characteristics. The new species, detected in NE Brazil, can be differentiated by their projections on the outer spore surface. Partial sequences of the LSU rRNA gene place both species next to *I. intraornata* in a monophyletic major clade related to *Gigasporaceae* and *Dentiscutataceae*.

Key words — *Glomeromycetes*, *Scutellospora*, molecular phylogeny, rDNA

Introduction

The phylum *Glomeromycota* has been extensively revised in recent years at all classification levels from class to species (e.g. Morton & Redecker 2001, Sieverding & Oehl 2006, Palenzuela et al. 2008, Goto et al. 2010, 2011, Oehl et al. 2011a,b,c). Major revisions were necessary within the order *Gigasporales* when separating *Gigasporaceae* species into four families (*Gigasporaceae*, *Scutellosporaceae*, *Racocetraceae* and *Dentiscutataceae*) and *Scutellospora* into seven genera when concomitant morphological and molecular spore analyses were undertaken (Oehl et al. 2008, 2010, 2011b,c). It was initially clear that *Dentiscutataceae,* in particular, was a heterogeneous family that needed more

detailed study (Oehl et al. 2008). Unfortunately, little progress has been made due to only a limited number of available isolates.

During recent studies on the diversity of the arbuscular mycorrhizal (AM) fungi (*Glomeromycota*) in Northeastern Brazil, *Dentiscutata colliculosa* B.T. Goto & Oehl, *Racocetra intraornata*, and two undescribed species were detected (Goto et al. 2009, 2010). All four species have introverted projections on the spore wall. The phylogenetic position of *R. intraornata,* however, remained unresolved while the two new species appeared to belong to the *Dentiscutataceae,* based on their spore formation on sporogenous cells and the presence of yellow brown to brown shields on the inner spore walls. Moreover, two major morphological characters separated these two new species within the *Dentiscutataceae* — the multiple-lobed structure of their openly organized shields and the presence of a tuberculate ornamentation on the inner surface of the hyaline middle spore wall. A multiple-lobed structure of openly organized shields has been detected only in the *Racocetraceae*, where shields are hyaline to sub-hyaline. Ornamentation on the inner surface of the middle wall is hitherto a unique character shared with no other species within the *Glomeromycota*. *Racocetra intraornata*, however, was understood to have a rather rudimentary germination shield where germ tube initiations are rarely found. The question therefore arose whether this species truly belongs to *Racocetra*, or might have a different phylogeny.

The main aim of this study was thus to analyze thoroughly and describe the two new species and to elucidate their phylogenetic position as well as that of *R. intraornata*.

Materials & methods

Study areas, soil sampling and soil parameters

One of the two new AM species was recovered in Serra da Jibóia, a fragment of the Atlantic Rainforest ('Mata Atlântica') in Santa Terezinha, Bahia State. The other species was found in several locations in the sand dunes of Mataraca, Paraíba State, (Souza et al. 2010, 2012) and in Natal, Rio Grande do Norte State. In the Serra da Jibóia, the soils were taken in September 2010, in Mataraca in January and August 2005, and in Natal in February 2010, as described in (Souza et al. 2010, 2012).

The Serra da Jibóia (12°51′S 39°28′W) is a forest fragment characterized by high plant diversity (Queiroz et al. 1996) located on a hillside surrounded by drier areas in transition to Caatinga (dry forest with trees and shrubs, deciduous during the dry season; Andrade-Lima 1964). Mean annual temperature is 17–28°C with 1200 mm of rain falling between April and July (Tomasoni & Santos 2003). The sand dunes in Mataraca are located at 6°28′–6°30′S 34°55′–34°57′W. The vegetation is typical of 'restinga,' a transition ecosystem between the primary coastal sand dunes and the tropical Atlantic Rainforest with physiognomy varying from tree-shrub to herbaceous plants (Oliveira-Filho & Carvalho 1993). The predominant geological formation is sandy-clay

sedimentary rocks, superimposed by fixed dunes. The climate is tropical-(semi-)humid (type Am of Köppen-Geiger, Kottek et al. 2006) with four months of dry season. Average annual temperature is 24–28°C and rainfall 1795 mm. The sand dunes in Natal represent one of the largest urban conservation areas with dune vegetation in Brazil. The 'Parque das Dunas' site (5°46′S and 35°12′W) also has typical 'restinga' vegetation. The climate is tropical rainy (type Am of Köppen) with a short dry period of four months. The mean annual temperature is 25.5 °C, and the mean annual precipitation is 1191 mm.

Soil pH (H_20) in Santa Terezinha was 4.6, organic C was 21.5 g kg^{-1} and available P (after Mehlich; Nelson et al. 1953) 2.0 mg kg^{-1}. In Mataraca, soil pH was 5.6, organic C 25.0 g kg^{-1} and available P 6.5 mg kg^{-1}. In Natal, pH was 5.6, organic C 16.6 mg kg^{-1}, and available P was 3.0 mg kg^{-1}. Soils in all three areas were sandy.

AM fungal bait cultures

The native AM fungal communities from Serra da Jibóia and Mataraca were cultured in bait (= trap) cultures over three consecutive cycles (three months each) using corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annuus* L.) in 500 mL pots, filled with autoclaved sand-vermiculite substrate (1:1 w/w; 400 g per pot) mixed with the natural field soil as AM fungal inoculum (50 g per pot), in the greenhouse of the Department of Mycology, Universidade Federal de Pernambuco, Recife. Additionally, multiple glomerospores of the new species were separated and used as infective propagules in single species cultures on *Sorghum bicolor* (L.) Moench as described in Palenzuela et al. (2010), Tchabi et al. (2010), and Goto et al. (2011). The new Serra da Jibóia species was successfully propagated in a two-species culture together with *Ambispora appendicula*, and the sand dune species in a bait culture together with *Racocetra tropicana* (Goto et al. 2011). However, no single species cultures were obtained from the two fungi. Spores isolated from the cultures or directly obtained from the field samples were used for morphological and molecular analyses.

Morphological analyses

Spores of the two new species and *R. intraornata* were extracted according to Sieverding (1991) and thereafter mounted in polyvinyl-alcohol–lacto–glycerin (PVLG), in PVLG + Melzer's reagent, and in water (Brundrett et al. 1994, Spain 1990). Over 100 spores were examined per species. The germination shields of each species were carefully separated from their spores by applying pressure and movements to cover slides in PVLG. Species descriptions utilize spore and germination terminology for gigasporalean species defined by Oehl et al. (2008, 2010, 2011b), Silva et al. (2008), and Goto et al. (2010, 2011).

Molecular analyses

DNA was extracted from single spores, with three single spore extractions for each of the two new species and one for *R. intraornata*. Individual spores were placed on a slide in a drop (5–10 µl) of ultrapure water, crushed with a needle, and used directly in the PCR reactions.

The extracts served as templates for a semi-nested PCR using primers ITS3 (White et al. 1990) – 28G2 (Silva et al. 2006) and LR1 (van Tuinen et al. 1998) – 28G2 consecutively. The template for the second PCR reaction was a 1:50 dilution of the first product. PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200

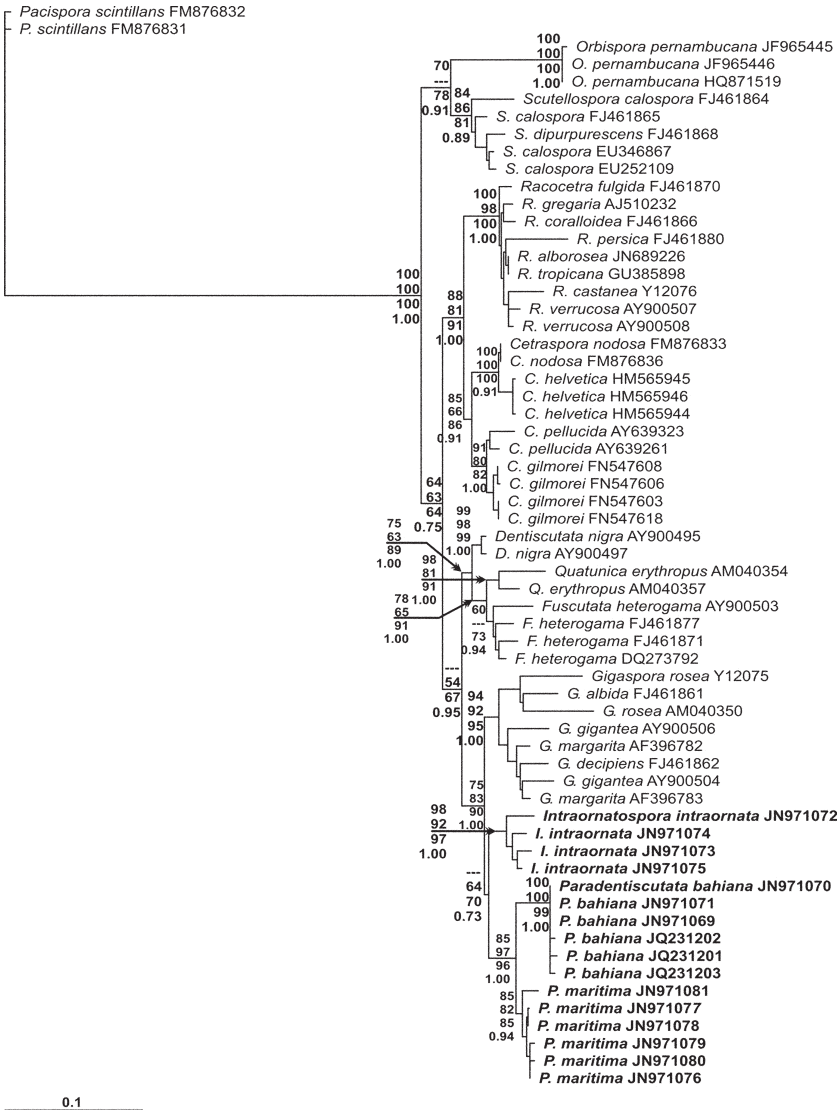


Fig. 1. Phylogenetic tree of the *Gigasporales* based on LSU rDNA analysis and rooted by *Pacispora scintillans*. Sequences are labeled with database accession numbers. Support values (from up to down) are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. *Intraornatospora intraornata*, *Paradentiscutata bahiana* and *P. maritima* sequences are in bold. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.53; Retention Index = 0.79).

mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1µM of each primer and 2 units of Taq™ DNA polymerase (Fermentas; Maryland, USA); cycling parameters were 5 min at 95°C (1 cycle), 45s at 94°C, 1 min at 55°C, 1 min at 72°C (40 cycles), and a final elongation of 7 min at 72°C followed the last cycle. The final amplicons (~690bp) were purified with a PureLink PCR Purification Kit (Invitrogen), sequenced directly or cloned with a CloneJET™ PCR Cloning kit (Fermentas; Carlsbad, USA) following the manufacturer's instructions and sequenced. Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil).

Through a BLASTn query of the National Center for Biotechnology Information databases, we verified that the sequences obtained from *P. maritima*, *P. bahiana,* and *R. intraornata* affiliated with the *Gigasporales* (*Glomeromycota*) before phylogenetic analysis. The AM fungal sequences (partial LSU rRNA) obtained were aligned with other glomeromycotean sequences from GenBank using ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). The sequences were deposited at GenBank under the accession numbers JN971069-JN971081 and JQ231201-JQ231203.

Maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications were performed using the Phylogenetic Analysis Using Parsimony (PAUP) vers. 4 (Swofford 2003). Bayesian (two runs over 1×10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The model of nucleotide substitution (GTR + G) was estimated using Topali 2.5 (Milne et al. 2004). Sequences from *Pacispora scintillans* were used as an outgroup.

Results

Molecular analyses

The phylogenetic analyses generated by the LSU rRNA gene sequences (Fig. 1) revealed that the three species grouped in a monophyletic major clade most closely associated with the *Gigasporaceae* and *Dentiscutataceae* clade, with 75% bootstrap value for at least one phylogenetic method. The data further confirmed that the new genera and two new species conferred bootstrap values above 79%, thus supporting a new family and two new genera within the *Gigasporales* (Oehl et al. 2011b). We hereafter describe the two new species in one new genus and transfer *R. intraornata* from *Racocetra* to another, hitherto monospecific genus as the type species of the new family.

Taxonomic analyses

Intraornatosporaceae B.T. Goto & Oehl, **fam. nov.**

MycoBank MB 563599

Sporae ad cellulas sporogeneas, tunicis duabus vel tribus; ornamentatione introvertita in superficie interiore tunicae mediae (tribus tunicis) vel exterioris (duabus tunicis); scutellum germinale multilobatum, flavum-brunneum ad brunneum in sporis tribus tunicis, hyalinum vel subhyalinum duabus tunicis.

Type species: *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto et al.

Sporocarps are unknown. Spores formed singly on bulbous sporogenous cells, terminally on a subtending hypha that arise from mycelial hyphae. Spores have 2 or 3 walls. The outer spore wall has 3–4 layers and inner wall 2–3 layers. Middle wall of 3-walled spores has an expanding outer and an inner layer with tuberculate ornamentation towards the inner wall. Outer wall of 2-walled spores also has introverted tuberculate ornamentation. Germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall. Shields of triple-walled spores yellow-brown to brown, with 4–8(–10) wave-like lobed projections; folds separate the lobes on the shield, and each lobe may have a germ tube initiation from where the germ tubes arise and penetrate the outer wall. Shield in bi-walled spores, hyaline to light yellow, with 4–8 wave-like lobed projections; folds separate the lobes on the shield, which often appears immature, even in more aged spores.

Type genus: *Intraornatospora* B.T. Goto et al.
Other genus: *Paradentiscutata* B.T. Goto et al.

Intraornatospora B.T. Goto, Oehl & G.A. Silva, **gen. nov.** Figs 2–4

MycoBank MB 563600

Sporae ornamentatione introvertita in superficie interiore tunicae exterioris; scutellum germinale superficie tunicae interioris, multilobatum hyalinumque.

Type species: *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto et al.

Etymology: Latin: *intra* (= inside), *ornata* (= ornamented), spora (= spore); referring to the position of the ornamentation on the spore in the type species.

Spores formed singly in soils, rarely in roots, on bulbous sporogenous cells that arise terminally on mycelial hyphae. The spores have two walls. The outer spore wall is generally triple-layered and continuous with the wall of the sporogenous cell. Inner surface of the outer spore wall has tuberculate or spiny projections. The inner wall is hyaline, 2–3 layered and forms de novo. A germination shield arises on the outer surface of the inner wall or beneath a thin outer layer of the inner wall; shield is hyaline to light yellow, generally oval to ellipsoid or subglobose, with several wave-like lobed projections forming the outer surface of the shield; folds separate the lobes on the shield. Germ tube initiations within the lobes often appear rudimentary and are rarely observed.

Intraornatospora intraornata (B.T. Goto & Oehl) B.T. Goto, Oehl & G.A. Silva, **comb. nov.** Figs 2–5

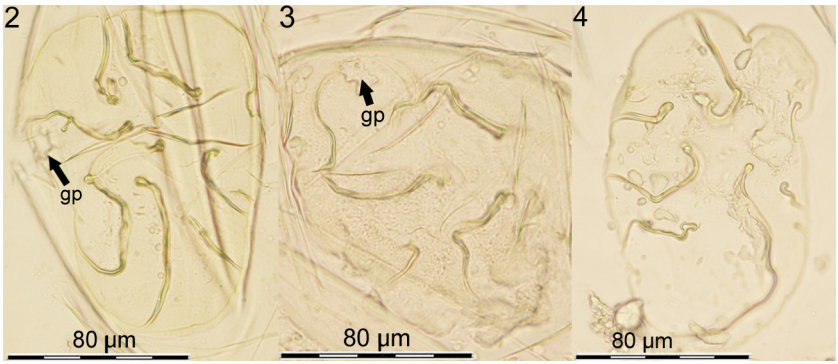
MycoBank MB 563601

≡ *Racocetra intraornata* B.T. Goto & Oehl, Mycotaxon 109: 485. 2009.

Paradentiscutata B.T. Goto, Oehl & G.A. Silva, **gen. nov.**

MycoBank MB 563602

Sporae ornamentatione introvertita in superficie interiore tunicae mediae; stratum exterius tunicae mediae expansivum; scutellum germinale, multilobatum, (flavo-)brunneum.



Figs 2–4. *Intraornatospora intraornata* (isotype, ZT Myc 775): typical, multiple-lobed germination shields; Germ pore (gp) as connection between shield and cell contents regularly visible when shield is in planar view (Figs 2–3); germ tube initiations rarely if ever visible.

Type species: *Paradentiscutata bahiana* Oehl et al.

Etymology: Latin: *para* (= equal), *dentata* (= dentate), *scutata* (= with shield); referring to the similarities with the germination shields of spores of *Dentiscutataceae*.

Sporocarps are unknown. Spores formed singly on bulbous sporogenous cells, terminally on subtending hypha that arise from mycelial hyphae. Spores have three walls; outer spore wall with 3–4 layers, middle wall with an expanding outer and a tuberculate ornamentation towards the inner wall, and an inner wall with 2–3 layers. Germination shields are yellow-brown to brown, as in *Dentiscutataceae*, but with 4–8(–10) wave-like lobed projections forming the outer surface of the shield; folds separate the lobes on the shield, and each lobe may have a germ tube initiation from where the germ tubes arise and penetrate the outer wall.

Paradentiscutata bahiana Oehl, Magna, B.T. Goto & G.A. Silva, **sp. nov.**

MycoBank MB 563603 Figs 5–20

Sporae aurantio-brunneae; stratum exterius verrucis, ≤ 1.0 µm in distantia.

Type: BRAZIL. Bahia State, Santa Terezinha, Serra de Jibóia, tropical rainforest fragment, [September 2010], [G.A. Silva], 92–9201 (**Holotype**, URM 83317); **Isotypes:** 92–9202 (URM 83318), 92–9203 (URM 83319), 9204–9212 (ZT Myc 7624).

Etymology: referring to Bahia State (NE Brazil), where the fungus was first found.

Glomerospores formed singly in soil, terminally on a subterminal bulbous suspensor cell (= 'sporogenous' cell). Glomerospores are dark orange brown to brown, globose, 190–260 µm in diameter, to subglobose, 190–265 × 190–240 µm, and have three walls (an outer, middle and inner wall). The spores become dark black brown to black when exposed to Melzer's reagent.

Outer wall (ow) is 3.6–6.0 µm thick in total and consists of 2(-3) layers: outermost wall layer (owl1) is hyaline to subhyaline to light yellow, semi-

persistent to persistent and 0.9–1.5 µm thick. It is covered with densely crowded irregular warts that are 0.8–2.0(–2.5) µm high and 1.0–2.1(–3.5) × 1.0–2.5(–5.0) µm wide. OWL2 is 2.1–3.4 µm thick, orange brown to brown, becoming dark orange brown to dark reddish brown when exposed to Melzer's reagent. OWL3 is concolorous and adherent with OWL2, very thin (0.6–1.1 µm), and generally not observed even in crushed spores.

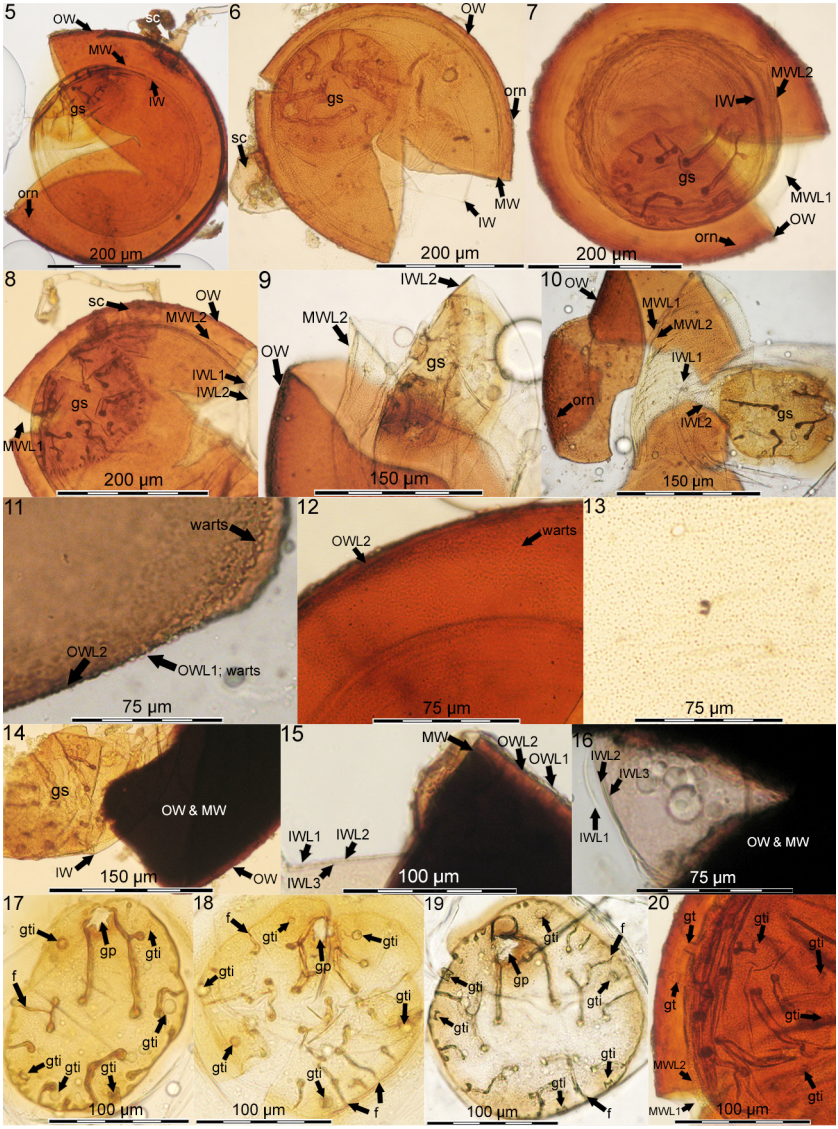
MIDDLE WALL (MW) is hyaline to rarely light yellow, bi-layered and 4.8–6.8 µm thick when mounted in water. MWL1 is hyaline and 3.8–5.2 µm thick when mounted in water. In crushed spores, it expands to 9.0–16.0 µm and regularly expands further to 20–32(–56) µm in lactic acid based mountants. MWL1 may even become completely transparent making it difficult to detect due to even stronger swelling processes. MWL2 is hyaline becoming light yellow in older spores, 1.0–1.6 µm thick and bears fine tubes on its inner surface that are 0.5–1.1 µm high, 0.4–1.0 µm wide and 1.0–2.5 µm spaced. MWL1 stains purple black to black in Melzer's reagent.

INNER WALL (IW) is triple-layered, 2.8–4.5 µm thick bearing a germination shield on the outer surface. The outer IW layer (IWL1) is hyaline, semi-flexible, 0.6–1.1 µm thick and often wrinkles in crushed spores when separated from IWL2. The second layer (IWL2) is semi-flexible to unite, rarely 'amorph' when slightly expanding in PVLG based mounting, and is 1.6–2.2 µm thick. The innermost layer (IWL3) is relatively thin (0.6–1.2 µm thick), flexible, mostly tightly adherent to IWL2, and therefore generally difficult to observe. IWL2 and IWL3 stain yellow-pink to light purple in Melzer's reagent.

SPOROGENOUS CELL (SC) is globose to elongate, 32–50 µm long and 28–38 µm broad. It is concolorous with the spore wall. Two wall layers are visible on the young sporogenous cell, which are continuous with OWL1 and with laminated OWL2. OWL1 at sc is 0.4–1.2 µm thick, smooth and semi-persistent; OWL2 is 1.0–2.5 µm thick and persistent as long as sc remains attached on the spore. One to two 'hyphal pegs' may be rarely detected on the sporogenous cells. The sporogenous hypha attached to the cell is also bi-layered, 12–21 µm in diameter, tapering to 7–11 µm within 100–250 µm distance from the sporogenous cell. Within this distance, the sporogenous hyphal wall tapers from 1.5–2.2 µm to 1.0–1.7 µm, and 4–9 septa originating from OWL2 may be visible in variable distances within the sporogenous hypha. The ornamentation on OWL1 does not continue on the wall of the sporogenous cell.

GERMINATION SHIELD is yellow brown to brown, subglobose to oval to cardioid, to rarely ellipsoid or oblong, 140–200 × 121–180 µm in diameter, and multiple-lobed, generally with (4–)6–10 lobes. The shield is open-organized, as

FIGS 5–20. *Paradentiscutata bahiana* (Type URM 83317–83319, ZT Myc 7624): **5–10**. Spores (crushed) formed on sporogenous cells (sc) with surface ornamentation (orn), showing outer wall (OW), middle wall (MW), inner wall (IW) and conspicuous, yellow brown to brown germination shields (gs). Each two layers of MW (MWL1-2) and IW (IWL1-2) visible. MWL1 expanding in lactic



acid based mountants; MWL2 with needle-tuberculate ornamentation. **11–12**. Verrucose surface ornamentation (warts) in cross and planar view, respectively. **13**. Ornamentation of MWL2 in planar view. **14–16**. Spore segments (crushed) in Melzer's reagent; expanding MWL1 stains dark purple to black purple. IW triple-layered (IWL1-3; IWL2 staining light purple to purple. **17–20**. Characteristic germination shields are open-organized with multiple wavy-lobes, mostly bearing, each, one germ tube initiation (gti) from where a germ tube (gt) emerges; lobes are separated by large folds (f), and a single germ pore (gp) connects the shield with the cell content.

known for species of *Racocetraceae*. Large folds (~11–45 µm long) arise from the shield wall separating the lobes. The one-layered shield wall and the folds are generally 1.2–1.8 µm thick. The shield periphery regularly appears slightly dentate until the germination has started. Each lobe may bear one rounded germ tube initiation (gti), 6.5–12.0 µm in diameter. The majority of the gti's may remain undetectable in young spores, becoming increasingly visible with age of spores. Single germination tubes may emerge from one, two to rarely three gti's during early germination, penetrating the OW and branching in the spore periphery within a short distance.

Arbuscular mycorrhiza forming (as proofed in dual-species cultures together with *Ambispora appendicula*, after staining in trypan blue).

Distribution: *Paradentiscutata bahiana* has been recovered only from natural tropical rainforest in Santa Terezinha, Serra da Jibóia, Bahia State.

Paradentiscutata maritima B.T. Goto, D.K. Silva, Oehl & G.A. Silva, **sp. nov.**

MycoBank MB 563604 Figs 21–31

Differt ad Paradentiscutata bahiana *in ornamentatione superficialis: stratum exterius papillis rotundatis, 3.0-8.0 µm in distantia.*

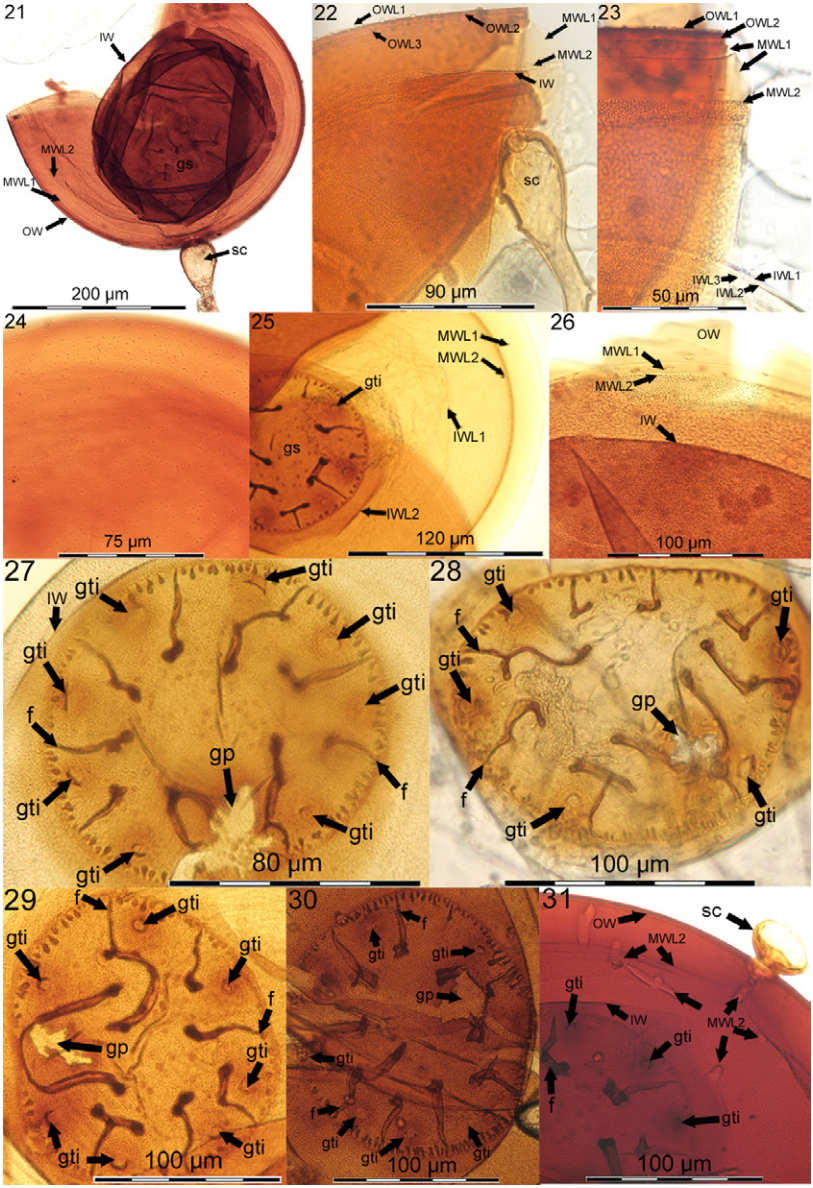
Type: BRAZIL. Paraiba State, Mataraca, Atlantic Rainforest biome, 'restinga' vegetation in sand dunes, [August 2005], [R.G. Souza], 93–9301 (**Holotype**, URM 83320); **Isotypes** 9302 (URM 83321), 9303 (URM 83322), 9304–9310 (ZT Myc 7625).

Etymology: Latin: *maritima* (=maritime, coastal), referring to occurrence in sand dune systems of the Atlantic coast.

Glomerospores formed singly in soils terminally on a subterminal or intercalary bulbous suspensor cell. Spores are globose (150–260 µm in diameter) to subglobose (145–250 × 165–280 µm), bright (yellowish) brown, orange brown to dark brown, with three walls: outer, middle, and inner (ow, mw, and iw).

Outer wall (ow) is three-layered: outermost wall layer (owl1) is 0.5–1.2 µm thick and has a papillae ornamentation with projections that are 1.5–2.5 µm high and 1.2–2.6 µm wide at base, and 3.0–8.0 µm apart. owl2 is yellow to orange, persistent, 2.5–3.8 µm thick and laminate. owl3 is 0.5–1.0 µm thick, tightly adherent to owl2 and especially difficult to distinguish in lactic acid-based mountants, also due to expansive mwl1 beneath. owl2 stains red to red-brown in Melzer's reagent. The straight pore channel at the spore base is about 2.5–4.2 µm broad and is generally closed by a plug formed by owl2.

Figs 21–31. *Paradentiscutata maritima* (Type URM 83320–URM83322, ZT Myc 7625): **21**. Spore (crushed) formed on sporogenous cell (sc) with three walls (ow, mw, iw) and germination shield (gs); iw staining purple in Melzer's reagent. Two layers of mw (mwl1-2) visible. **22–23**. Spore wall structure: ow triple-layered (owl1-3), bi-layered mw, and triple layered iw (iwl1-3); spore surface ornamentation of owl1, with dispersed, blunt papillae. **24**. Blunt ornamentation in planar view. **25–26**. mwl1 expanding in lactic acid based mountants; mwl2 with needle-tuberculate



ornamentation. Yellow brown to brown germination shields with germ tube initiations (gti). **27–30**. Germ shields (planar view) with 4–8 lobes, mostly bearing, each, one germ tube initiation (gti) from where germ tubes (gt) emerge; single lobes are separated by large folds (f), and a single germ pore (gp) connects the shield with the cell content. **31**. gti of the germ shield partly visible on MWL2.

Middle wall (mw) is hyaline to rarely light yellow, bi-layered and 5.2–7.6 (–8.9) µm thick when mounted in water. mwl1 is hyaline, and 4.0–5.1 µm thick in water. It expands to 7.5–14.6 µm in crushed spores and regularly expands further to 20–28(–51) µm in lactic acid based mountants. mwl2 is hyaline to light yellow, 1.2–2.5(–3.8) µm thick, and densely packed with small tuberculate-warty projections on the inner surface. Projections are 1.0–1.5(–2.5) µm long and 0.5–1.0(–1.1) µm broad. Warts are about (0.5–)1.0 (–2.5) µm apart from each other.

Inner wall (iw) is triple-layered bearing a germination shield on the outer surface. Outer layer of the inner wall (iwl1) is hyaline, semi-flexible and 0.5–1.2 µm thick. Second layer (iwl2) is unit, semi-flexible and 1.2–2.5 µm thick. Innermost layer (iwl3) is thin (0.4–0.8 µm thick), flexible, generally tightly adherent to iwl2, and difficult to observe. iwl2 stains reddish-brown to purple in Melzer's reagent.

Sporogenous cell is subglobose to elongate, concolorous with the spore, or slightly lighter in color than the spore, and 46–64 µm long and 71–76 (–79) µm broad. Two wall layers are generally visible on the sporogenous cell, continuous with owl1 and owl2. owl1 on the sporogenous cell is about 0.5–1.6 µm; adherent owl2 is about 2.5–7.6(–10.2) µm thick. The ornamentation on owl1 does not continue on the wall of the sporogenous cell. The pore of the sporogenous cell is generally closed at the septum to the attached 'sporogenous hypha' by a septum arising from owl2.

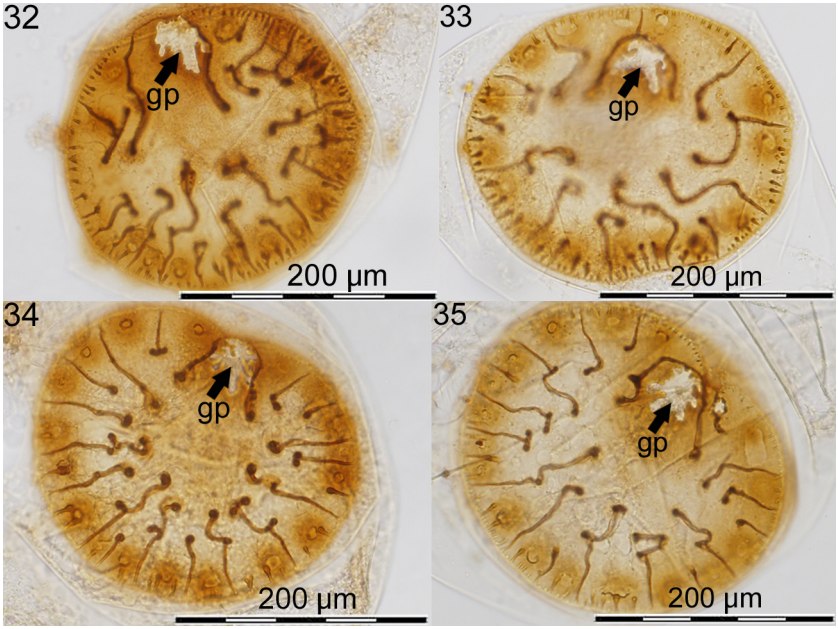
Germination shield is yellow-brown to brown, cardioid to oval or ellipsoid, 181–189 × 166–171 µm in diameter, and has several 4–8(–10) lobes, that are easy to differentiate as they are often wavy in planar view and separated by large folds. These folds (about 5–15 µm long) arise from the shield wall. The one-layered shield wall and the folds are generally only 0.5–1.7 µm thick. Each lobe regularly bears one rounded germ tube initiation, 1.2–2.5 µm in diameter, from where the germination tubes emerge.

Additional material examined: **BRAZIL. Rio Grande do Norte**, Natal, from sand dunes (URM 83483–83485).

Distribution: *Paradentiscutata maritima* has been recovered from two sand dune locations in NE Brazil (Mataraca, Paraíba State, and Natal, Rio Grande do Norte State).

Spore development in *Intraornatospora* and *Paradentiscutata*

In *I. intraornata* the outer spore wall is continuous with the wall of the sporogenous cell and the attached sporogenous hypha. The ornamentation on the inner surface of the outer spore wall differentiates during spore formation. The inner wall forms de novo during spore formation like an 'endospore'. In *Paradentiscutata* species, the outer wall is also continuous with the wall of the sporogenous cell and the sporogenous hypha attached. Although uncertain



Figs 32–35. *Dentiscutata biornata* (isotype, OSC 49583): Typical germination shields have one germ pore (gp) 12–24 compartments and 12–24 germ tube initiations (gti); compartments (dense lobes) separated by large folds; shield periphery is dentate.

whether the middle wall forms completely de novo or differentiates from a thin layer present on the inner surface of the outer wall, we did observe that during spore formation MWL1 differentiates first, showing several laminae when exposed to PVLG and other lactic acid based mountants. MWL2 differentiates on the inner surface of developed MWL1. Thereafter, IW is formed de novo, and, in the final stage of spore development the germination shield differentiates on the outer IW surface.

Discussion

Distinct spore morphologies were recognized for both *Paradentiscutata* and *Intraornatospora*. *Paradentiscutata* spp. have three-walled spores with a diagnostic tuberculate ornamentation on the inner surface of the middle wall and an openly organized, multiple-lobed, yellow-brown to brown germination shield on the outer IW surface. *Intraornatospora intraornata* has a tuberculate ornamentation of the inner OW surface, and on the outer IW surface a multiple lobed, hyaline to light yellow germination shield that regularly appears to be rudimentary even in older spores, since clearly visible germ tube initiations have rarely been found (Figs 2–4). Germination itself has yet to be observed in spores

of this species, so we do not know whether *I. intraornata* germinates through the rudimentary germ shield or directly from the warty inner projections of the outer wall (as in *Gigaspora*). The two new genera, morphologically somewhat dissimilar, might better be separated into two families. However, the database so far is too small to make a final conclusion on this aspect.

The two *Paradentiscutata* spp. can easily be distinguished through their ornamentation on the outer spore surface, a character that clearly separates species in other glomeromycete families (e.g., *Racocetra verrucosa* and *R. persica*, *Dentiscutata nigra* and *D. reticulata*, *Acaulospora scrobiculata* and *A. cavernata;* Oehl et al. 2008, 2011d). Of the *Gigasporales* species, *P. maritima* resembles only *Dentiscutata biornata*, which differs in a compact germination shield with multiple (12–24) compartments in mature spores (Figs 32–35) that are not openly organized or multiple-lobed (Spain et al. 1989) as in *P. maritima*, which has 4–8(–10) lobes. Additionally, the double ornamentation of *D. biornata* belongs to the outer spore wall according to Spain et al. (1989). These important morphological differences point out species dissemblance and support the new speciation. However, further research may consider checking the phylogenetic relationships between *D. biornata* and the two new species.

Species of *Intraornatosporaceae*, *Dentiscutataceae,* and *Gigasporaceae* are predominantly found in warmer climates. The *Intraornatosporaceae* have so far been detected only in tropical areas, while *Dentiscutataceae* spp. are known also from sub-tropical and Mediterranean regions and *Gigasporaceae* spp. have at times been recovered from temperate regions (Jansa et al. 2002, Oehl et al. 2010). It is possible that *Dentiscutataceae* and *Gigasporaceae* may have evolved to cooler periods and climates than *Intraornatosporaceae*. Further AM fungal diversity research will also help verify this hypothesis.

Acknowledgments

The authors acknowledge, in special, the valuable comments and revisions of several experts on the manuscript and appreciate the corrections by Shaun Pennycook, Nomenclatural Editor, and suggestions by Lorelei L. Norvell, Editor-in-Chief. This work was supported by Protax (Program of Capacitation in Taxonomy), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), which provided a research grant to Leonor C. Maia and scholarships to Danielle K. Alves da Silva and Daniele M.A. de Assis; Programa de Biodiversidade do Semi-árido (PPBio); FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) which provided financial support to G.A. Silva; Universidade Federal de Pernambuco (UFPE), which provided a grant to F. Oehl as 'visiting professor.' The authors would like to thank the Millennium Inorganic Chemicals Mining, a Cristal Company, for logistical support.

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